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METAM SODIUM REDUCES VIABILITY AND INFECTIVITY OF *EIMERIA* OOCYSTS

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ABSTRACT: Metam sodium (MS, sodium N-methyldithiocarbamate) is a widely used soil pesticide. Fumigation or chemical sterilization of poultry litter containing infectious oocysts could be an effective strategy to block the transmission of avian coccidia. In the current study, the effect of MS on the viability and infectivity of ocysts was investigated. The development of isolated, unsporulated oocysts of both *Eimeria tenella* and *Eimeria maxima* was inhibited, in a dose-related manner (IC_{50} 8 to 14 μg/ml), by exposure to aqueous MS. Most treated oocysts failed to develop beyond early stages of sporulation. To determine the effect of MS on infectivity, isolated oocysts of *E. tenella*, *Eimeria acervulina*, and *E. maxima* were exposed for 24 hr to aqueous concentrations of MS ranging from 0 to 1,000 μg/ml. Treated oocysts were inoculated into chickens, and parameters of coccidiosis infection were compared to chickens inoculated with equal numbers of untreated oocysts. In a dose-related manner, MS significantly reduced the infectivity of oocysts with maximum effect observed at a dose of 300 μg/ml. When a mixture of oocysts containing 3 coccidian species was exposed to 300 μg/ml MS, from 0 to 24 hr, infectivity of oocysts was significantly reduced after a minimum of 12 hr of exposure. Treatment of aqueous slurries of litter samples obtained from commercial poultry houses, with 300 μg/ml MS for 24 hr, prevented the sporulation of eimerian oocysts in the litter samples relative to untreated control samples. The results indicate that MS could be used to reduce coccidial contamination of poultry litter.

Coccidiosis, caused by *Eimeria* spp., remains a major disease of poultry that produces significant losses due to morbidity, mortality, and decreased productivity (Allen and Fetterer, 2002). The primary interventions used to reduce losses due to coccidiosis are prophylactic drug treatments, administered in the feed or water, and the use of live or attenuated vaccines (Allen and Fetterer, 2002; Shirley et al., 2007). Both drugs and vaccines target the invasive stages within the bird and attempt to prevent or reduce the impact of disease by reduction in the pathology caused by these invasive stages.

Sporulated oocysts contaminating the poultry litter are the infectious stage of poultry coccidia. Unsporulated oocysts are passed in the feces of infected birds and subsequently sporulate to the infective stage in the litter. The oocysts are clearly essential for development of the disease, causing both the onset of the disease and proliferation of the disease within a poultry facility. There are a number of reports of disinfecting strategies to reduce environmental contamination by other coccidia of veterinary and human health importance (King and Monis, 2007; Wainwright et al, 2007; Dumetre et al., 2008). In spite of the importance of the oocysts to the epidemiology of poultry coccidian, there is limited information about the use of sanitizing agents or chemical disinfectants to reduce the viability or infectivity of eimerian oocysts in the environment. However, one study reported the development of bioassays to investigate the efficacy of disinfectants in reducing the viability of *Eimeria tenella* oocysts (Duagschies et al., 2002).

Metam sodium (MS, sodium N-methyldithiocarbamate) is a widely used compound generally applied to soil as a pre-emergent herbicide, fungicide, insecticide, and nematicide (Kiely et al., 2004; Ingham et al., 2007). Surveys indicate that MS is the third, most-widely used conventional pesticide, with an annual use of more than 60 million pounds (Kelly et al., 2004). The mode of action of MS is not clearly understood, but it is rapidly converted to the active compound methylisothiocyanate (MITC), which has a number of biological activities most-likely mediated by interaction of MITC with free amino groups on proteins (Mathiessen and Shackleton 2005; Tilton and Tanguay, 2008).

Although widely used as a soil pesticide, the ability of MS to alter the viability and infectivity of oocysts has not been evaluated. The present study examines the ability of MS to reduce the viability and the infectivity of three important species of avian coccidia.

MATERIALS AND METHODS

Chickens

All chickens were 1-day-old SexSal cockerels (White Rock × Rhode Island Red) obtained at 1 day of age (Hyline Hatchery, Elizabethtown, Pennsylvania). The chickens were raised in brooders for 2 wk and then housed in suspended wire cages (91 cm long, 61 cm wide, 27 cm high) with 5 chickens per cage. The birds had access to water ad libitum and were fed a broiler starter diet. Housing temperature of caged birds ranged from 25 to 28 C under 24 hr of continuous lighting. At between 3–4 wk of age, the birds were used for experiments.

Parasites

Oocysts of the avian coccidia, *E. tenella* (Wampler isolate), *Eimeria acervulina* (#12 isolate), and *Eimeria maxima* (Tyson isolate), were maintained at the Animal Parasitic Disease Laboratory by periodic infection of parasite-free chickens. Oocysts were collected and maintained as previously described (Fetterer and Barfield, 2003).

Viability of oocysts

Unsporulated oocysts were harvested from feces of chickens infected with either *E. tenella* or *E. maxima*. Oocysts were isolated from fecal material by flotation on saturated salt and washed 3–4 times with water. Samples were kept at 4 C throughout the isolation procedure to prevent sporulation. An aqueous suspension of isolated, unsporulated oocysts was added to wells of 24-well plates (Becton Dickinson, Lincoln Park, New Jersey) in a 0.5 ml volume to give 5×10^5 oocysts/well. At time 0, 10 μl of freshly made aqueous dilutions of MS stock (51% aqueous solution; AMVAC Chemical Corporation, Los Angeles, California) were added to each well to give final concentrations ranging from 1 to 1,000 μg/ml. Control wells (0 μg/ml MS) received 10 μl of water. Plates containing oocysts were incubated at 28 C for 24 hr in a shaking water bath. After incubation, plates were examined with an inverted microscope, and the number of sporulated oocysts in 100 total oocysts from each well was determined. Results were expressed as percent sporulation.

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Effect of MS on oocysts output

To determine the dose response of MS on infectivity of sporulated oocysts, 10 ml of aqueous suspensions of isolated oocysts of *E. tenella*, *E. acervulina*, or *E. maxima* were placed in 50-ml conical tubes. Aqueous dilutions of MS were added in 100-µl volumes to give final MS concentrations between 3 and 1,000 µg/ml. Control tubes (0 µg/ml) received 100 µl of water. Tubes were placed on a platform rocker and incubated for 24 hr at 22 C. Tubes were centrifuged, supernatant removed, and oocysts resuspended in water. Wash procedure was repeated for a total of 3 washes. Chickens were wing-banded, weighed, and randomly assigned to treatments groups (3 chicks per cage, 9 chicks per group). The treatment groups consisted of chicks receiving oocysts treated with 0, 3, 10, 30, 100, 300, and 1,000 µg/ml MS and a group that was not infected (uninfected control group). On day 1, chicks were infected by oral gavage with either 150,00 *E. tenella*, 300,000 *E. acervulina*, or 20,000 *E. maxima* oocysts. On day 7, chicks were weighed, killed by cervical dislocation, and lesion scores (LS) from the predilection site for each species was determined as a single-blind study, as previously described (Johnson and Reid, 1970). Feces from each cage of 3 birds were collected for 24 hr prior to termination of experiment. Feces were homogenized in water and aliquots were diluted and counted with a hemocytometer. Results were expressed as oocysts/24 hr.

To determine the time course of MS on infectivity of oocysts, an aqueous dilution of MS was added to 50-ml conical tubes containing 10 ml of an aqueous suspension of a mixture of *E. tenella*, *E. acervulina*, and *E. maxima* oocysts to give a final concentration of 300 µg/ml. Tubes were incubated on a platform rocker for 0 (untreated), 1, 3, 6, 12, and 24 hr at 22 C. Following the appropriate incubation period, the suspensions of oocysts were centrifuged, the supernatant removed, and oocysts resuspended in 50 ml of water. This procedure was repeated for a total of 3 washes. Chickens were wing-banded and randomly allocated to treatment groups (3 per cage, 9 per group). Treatment groups consisted of chickens receiving oocyst mixtures treated with 300 µg/ml MS for 0, 1, 3, 6, 12, or 24 hr. An additional treatment group consisted of uninfected control chickens. Chicks in the infected groups were inoculated by oral gavage on day 1 with an oocyst mixture that contained 100,000 *E. tenella*, 200,000 *E. acervulina*, and 15,000 *E. maxima* oocysts. On day 7, chickens were weighed, killed by cervical dislocation, and LS determined on the anterior gut, mid gut, and cecae from each bird. Feces from each cage of 3 birds were collected for 24 hr prior to termination of experiment and fecal counts determined as described above.

Effect of MS on viability and oocysts output in poultry litter

Litter samples were obtained from 3 commercial poultry farms from the southeastern United States. Before use, samples were held at 4 C for 2–3 wk. Two, 10-g samples of litter from each farm were homogenized with water in a blender to give a total volume of 50 ml. The number of oocysts in each sample ranged from 2–8 $\times$ 10^4 oocysts/ml. By microscopic examination, the oocysts were determined to be primarily unsporulated *E. maxima* and *E. acervulina*. MS was added to 1 slurry sample from each farm to give a final concentration of 300 µg/ml. The other sample from each farm was untreated and served as a control. All samples were incubated, with stirring, for 72 hr at 22 C, and samples were examined microscopically to determine the percentage of sporulated oocysts. Results were expressed as percent sporulated oocysts. To determine the infectivity of these oocysts, each sample of the treated oocysts, and the corresponding untreated control from each, were administered to a group of 3 chickens by top-dressing fecal slurry onto feed. For a 24-hr period beginning on day 6 post-infection, feces were collected from each cage of birds and the total number of oocysts produced per 24 hr was determined as described above.

Statistical analysis

Except for LS, values were expressed as means and the error bars represent 1 SEM. The LS were expressed as median values, analyzed with a Kruskal-Wallis analysis of variance on ranks, and values were compared with Dunett's *t*-test. Other data were analyzed by an analysis of variance with Bonferroni's *t*-test, which was used to determine the differences between means (Systat Software, Inc., San Jose, California). Values were considered significant at $P < 0.05$. Curves were fitted to data using nonlinear regression. The value for concentrations of MS giving a 50% inhibition of sporulation (EC_{50}) was calculated from curve fitting to data using a 4-parameter logistic model (Sigma Plot, Systat Software, Inc.).

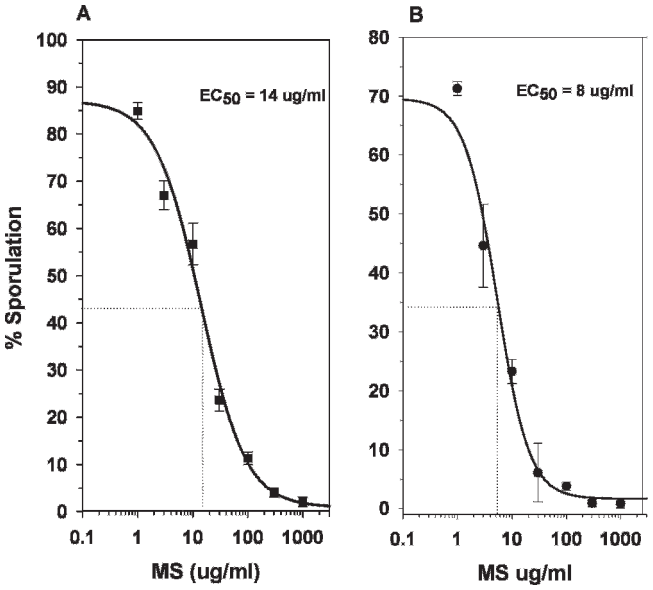

FIGURE 1. The dose–response effect of metam sodium (MS) on sporulation of (**A**) *E. tenella* and (**B**) *E. maxima* oocysts. Values are expressed as means (n = 4) and error bars represent 1 SEM. The curves were fitted to the mean values by nonlinear regression. Values for EC_{50} represent the concentration of MS that inhibits 50% of the maximum sporulation as calculated from the curve-fitting program. The dashed lines on each plot graphically represent the EC_{50}.

RESULTS

Effect MS on viability of oocysts

Incubation of unsporulated oocysts in MS inhibited subsequent sporulation in a dose-related manner for both *E. tenella* (R^2 = 0.97) and *E. maxima* (R^2 = 0.96). Sporulation in the absence of MS was 88% and 70%, for *E. tenella* and *E. maxima*, respectively (Fig. 1). The EC_{50} for MS treatment was 14 µg/ml for *E. tenella* oocysts (Fig. 1A) and 8 µg/ml for *E. maxima* oocysts (Fig. 1B). For both species, MS concentrations between 300 and 1,000 µg/ml gave the maximum effect on sporulation, while 1 µg/ml was without effect.

Effect of MS on oocysts infectivity

For all 3 species, there was a significant curve linear relationship (second-order polynomial, $R^2 > 0.98$) between the concentrations of MS applied to oocysts and the weight gain of chicks infected with those oocysts (Fig. 2A). The concentration of MS that gave the maximum weight gain relative to untreated control was about 100, 300, and 1,000 µg/ml, for *E. tenella*, *E. acervulina*, and *E. maxima*, respectively.

The 24-hr oocyst output of birds infected with MS-treated oocysts, from all 3 species, was reduced relative to untreated–infected birds (Fig. 2B). For birds infected with *E. maxima*, there was a curve linear relationship (second-order polynomial, R^2 = 0.98) between MS concentration and oocyst output of infected birds. There was not a significant curve linear relationship between the MS concentration and oocyst output of birds infected with *E. acervulina*. However, pretreatment of oocysts with MS at

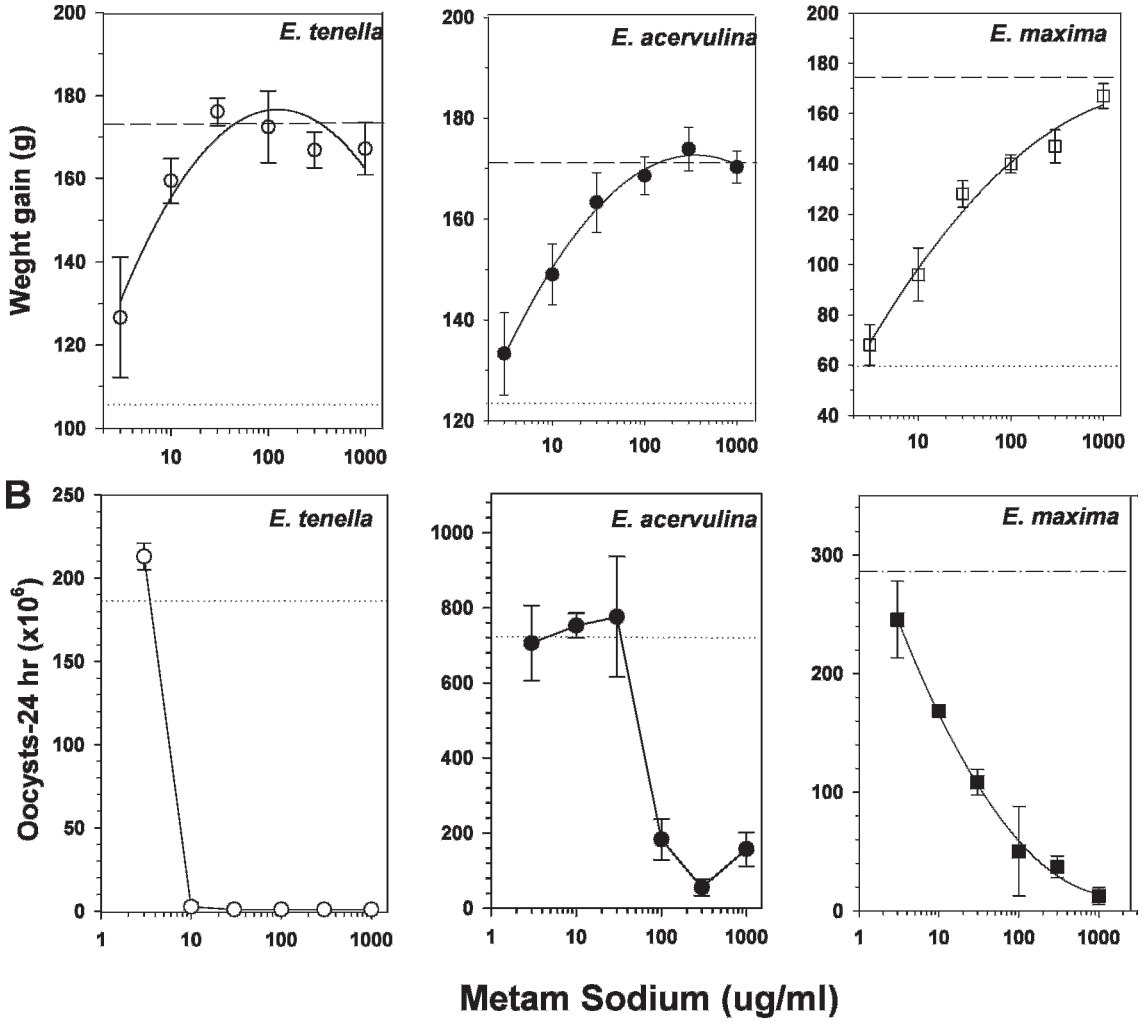

FIGURE 2. The effect on (**A**) weight gain and (**B**) oocyst production of chicks infected with *E. tenella*, *E. acervulina*, or *E. maxima* oocysts treated with increasing concentrations of metam sodium (MS). Values are means (n = 9) and error bars represent 1 SEM. If possible, curves were fitted to data using nonlinear regression. The dashed lines represent mean values for uninfected, untreated chickens. The dotted lines represent the mean values for infected, untreated chickens.

concentrations greater than 100 μg/ml significantly reduced the oocyst output. In contrast to both *E. acervulina* and *E. maxima*, the oocyst output of birds infected with *E. tenella*-pretreated oocysts was greatly reduced using as little as 10 μg/ml MS.

The LS of birds infected with oocysts pretreated with MS were reduced with increasing MS concentration (Fig. 3). No lesions were observed in birds infected with *E. tenella* and *E. acervulina* at MS concentrations 100 μg/ml and higher. Although lesions were observed in chickens infected with MS-treated *E. maxima* oocysts, LS were significantly reduced compared to those observed in chickens infected with untreated oocysts. The LS of infected, untreated birds were 3, 3, and 4, for *E. tenella*, *E. acervulina*, and *E. maxima*, respectively.

The weight gain, LS, and oocyst output of birds infected with oocysts treated with 300 μg/ml MS was dependent on time of oocyst exposure to MS (Fig. 4). The weight gain of birds infected with MS-treated oocysts was equal to control values after 12 hr of oocyst exposure (Fig. 4A). Lesions were absent (LS = 0) from the mid gut and cecae after oocysts were exposed to MS for 12 hr, while lesions of the anterior gut were absent only after 24 hr exposure to MS (Fig. 4B). The oocyst output of birds infected with treated oocysts declined in a time-dependent manner (Fig. 4C), with oocysts being significantly reduced by about 80% relative to infected, untreated controls after 24 hr exposure to MS. However, birds infected with oocysts exposed to MS for 24 hr still produced about 200 million oocysts/24 hr.

Effect of MS on oocysts in litter samples

Addition of 300 μg/ml MS to litter samples from 3 commercial poultry farms greatly diminished the sporulation of oocysts (Fig. 5A). The percentage of oocysts that sporulated ranged from 28 to 48% in untreated samples, while treatment of litter samples with MS reduced the sporualtion to less than 3%. The oocyst output of birds fed treated oocysts was reduced (>93%) compared to oocyst output of birds fed untreated oocysts (Fig. 5B). However, oocysts ($>10^6$ oocysts/24 hr) were still present in the feces.

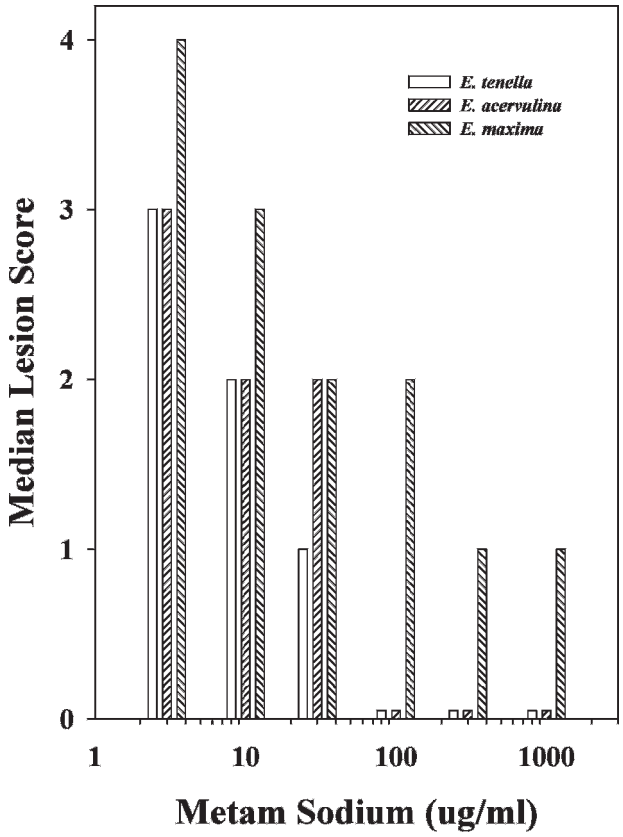

FIGURE 3. The effect on lesion scores (LS) of chicks infected with *E. tenella*, *E. acervulina*, or *E. maxima* oocysts treated with metam sodium (MS). Values are medians (n = 9).

DISCUSSION

In the present study, viability is defined as the ability of oocysts to sporulate normally, and infectivity is defined as the ability of sporulated oocysts to elicit a normal coccidia infection in naïve chickens. The current results clearly demonstrate that exposure of oocysts to MS at a concentration ≥300 μg/ml for more than 12 hr reduces the viability and infectivity of oocysts of 3 important species of *Eimeria*. Several observations in the present study support this conclusion.

MS inhibited sporulation of both *E. tenella* and *E. maxima* oocysts in a dose-dependent manner, with *E. maxima* having a slightly lower EC_{50} than did *E. tenella*. Because *E. acervulina* oocysts develop very quickly after shedding, and are more difficult to isolate rapidly from feces, they were not used in these experiments. MS, or its active component MITC, must penetrate the oocyst wall to exert its activity. However, the oocyst wall contains highly cross-linked proteins (Belli et al., 2003), which impart environmental stability, and is thought to be impermeable to many solutes. Therefore, it was somewhat surprising that MS is able to prevent sporulation at relatively low concentrations. MITC has a low molecular weight (73.1 g/mole) and may be converted to a gaseous state upon contact with soil. Therefore, MITC may diffuse across the oocyst wall, as has been demonstrated for urea and some gases (Jensen et al., 1976).

Since weight loss is reversed, and oocyst output and lesions scores are lowered, by pretreatment of oocysts with MS, it apparently reduces infectivity of eimerian oocysts by inactivating the sporozoites within sporulated oocysts. The reduction in weight gain caused by infection is clearly related to the MS concentration applied to the oocysts. There appears to be some

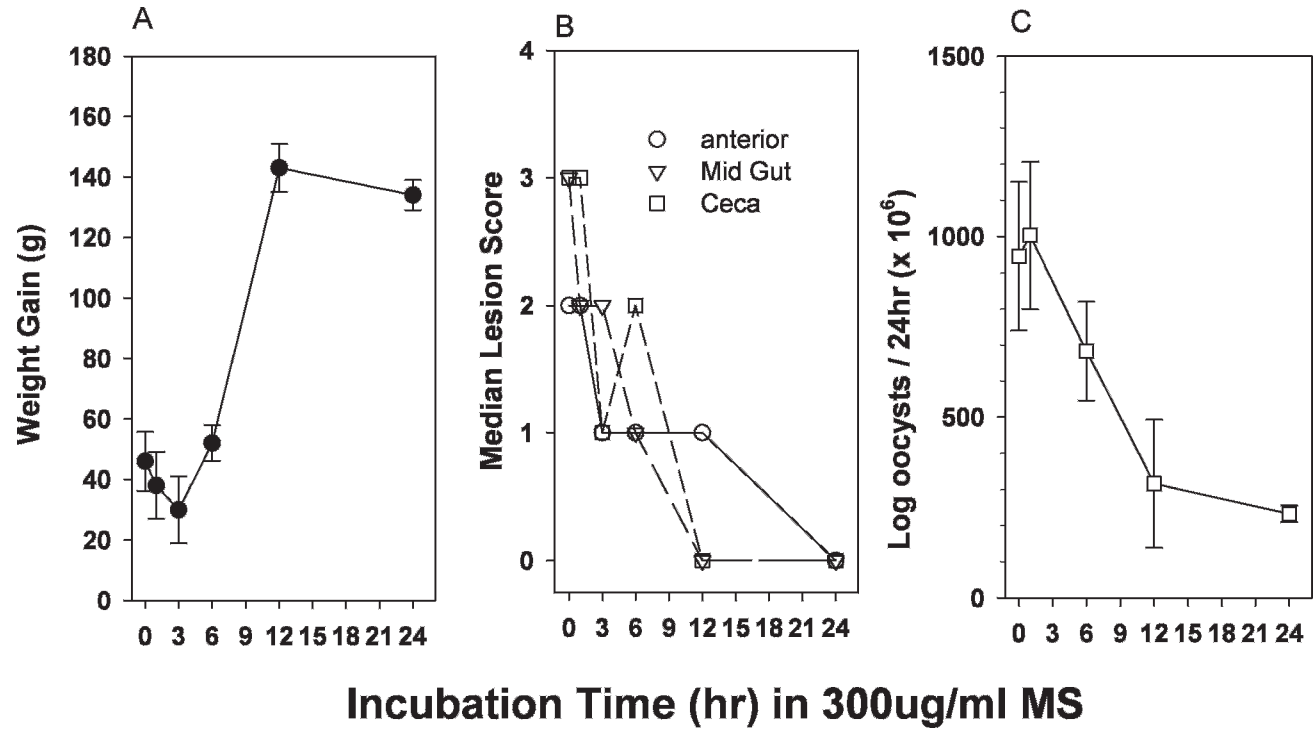

FIGURE 4. The (**A**) weight gain, (**B**) oocyst output, and (**C**) lesion score (LS) of chicks infected with a mixture of *E. tenella*, *E. maxima*, and *E. acervulina* oocysts treated for various time periods with metam sodium (MS). For weight gain and oocyst output, values are means (n = 9) and error bars represents 1 SEM. For LS, values are medians (n = 9).

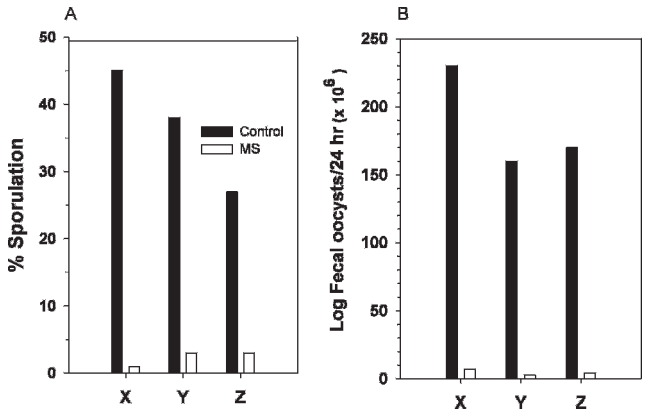

FIGURE 5. The effect of 300 μg/ml metam sodium (MS) on *Eimeria* oocyst viability and infectivity in slurry of feces collected from 3 different poultry farms. (**A**) The effect of MS on sporulation of oocysts in fecal slurries from 3 farms (X, Y, Z). Values are from replicate counts from a single slurry for each treatment. (**B**) Fecal oocyst counts from cages of chicks (3 per cage) fed fecal slurries treated or untreated with 300 μg/ml MS. Values represent the 24-hr oocyst output for each cage (3 birds).

species differences at the lower concentrations of MS, with *E. tenella* oocysts being most sensitive compared to *E. acervulina* and *E. maxima*. However, *E. tenella*, *E. maxima*, and *E. acervulina* oocysts treated with MS concentration ≥300 μg/ml were incapable of depressing weight gain. There were also species differences with respect to LS. The LS of birds infected with MS-treated *E. maxima* oocysts are reduced to a lesser extent than those of *E. acervulina* or *E. tenella*. The reason for these differences is not clear, but the strain of *E. maxima* used is quite virulent, producing LS of 4 for the birds infected with untreated oocysts compared to LS of 3 for the other 2 species. *Eimeria tenella* oocysts also appear to be the most sensitive to pretreatment with MS, in regard to oocyst output of infected chickens. MS treatment of *E. acervulina* and *E. maxima* oocysts with MS concentrations ≥300 μg/ml significantly reduces oocyst output of infected birds, but even at these concentrations, relatively large numbers of oocysts still remain in the feces. It seems that enough sporozoites within the oocysts survive the MS treatment to produce significant numbers of oocysts. Due to the asexual reproduction that occurs during schizont stages, there is a nonlinear relationship between oocyst output and number of infective oocysts ingested. Modeling studies with *E. maxima* indicate an approximately quadratic relationship between oocysts ingested and oocysts output, and the maximum fecundity is about 3.8×10^4 oocysts for each oocyst ingested (Johnston et al., 2001). Therefore, when ingested by birds, even a small percentage of oocysts surviving MS treatment can result in a relatively large oocyst output.

The current data indicate that the minimum time of exposure of MS required for inactivation of oocysts containing a mixture of species is about 12 hr. This conclusion is supported by the reduction in weight loss and by the LS that are observed between 12 and 24 hr of exposure. This length of exposure is most-likely needed for MS or MITC to diffuse into the oocysts. Although the oocyst output was significantly reduced, it remained relatively high in birds infected with oocysts exposed to MS for 24 hr. This suggests that a longer exposure, a higher concentration, or both may be needed to eliminate oocyst output completely.

Practical application of MS in reducing the viability and infectivity of oocysts requires that an agent such as MS must be compatible with application to oocyst-contaminated poultry litter. Our observation on the activity of MS on oocysts in litter samples obtained from commercial poultry farms, although preliminary, suggests that MS retains activity in poultry litter. Exposure of oocysts in litter to 300 μg/ml MS for 72 hr greatly reduces the sporulation and infectivity of the oocysts. However, the presence of reduced oocyst numbers indicates that treatment of the litter was not sterilizing in nature.

Together, the current results indicate that MS is quite effective in preventing sporulation and in reducing infectivity of eimerian oocysts. It is possible that MS could be used in a strategy to sterilize poultry litter between grow-out periods for broiler chicks. The use of MS has the added benefit of being active against a wide range of micro-organisms and could simultaneously reduce viral and bacterial contamination in litter. More extensive trials are needed, utilizing a system simulating normal poultry production, in order to determine the ultimate feasibility of MS in the control of oocysts in poultry litter.

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LITERATURE CITED

ALLEN, P. C., AND R. H. FETTERER. 2002. Recent advances in biology and immunobiology of *Eimeria* species and in diagnosis and control of infection with these coccidian parasites of poultry. Clinical Microbiological Reviews **15:** 58–65.

BELLI, S. I., M. G. WALACH, C. LUXFORD, M. J. DAVIES, AND N. C. SMITH. 2003. Roles of tyrosine-rich precursor glycoproteins and dityrosine- and 3,4 phenylalanine-mediated protein cross-linking in development of the oocysts wall in the coccidan parasite *Eimeria maxima*. Eukaryotic Cell **2:** 456–464.

DAUGSCHIES, A., R. BOSE, J. MARX, K. TEICH, AND K. T. FRIEDHOFF. 2002. Development and application of a standardized assay for chemical disinfection of coccidia oocyst. Veterinary Parasitology **103:** 299–308.

DUMETRE, A., C. LE BRAS, M. BAFFETT, P. MENECEUR, J. P. DUBEY, F. DEROUIN, J. P. DUGUET, M. JOYEUX, AND L. MOULIN. 2008. Effects of ozone and ultraviolet radiation treatment on the infectivity of *Toxoplasma gondii*. Veterinary Parasitology **153:** 209–213.

FETTERER, R. H., AND R. C. BARFIELD. 2003. Characterization of developmentally regulated oocyst protein from *Eimeria tenella*. Journal of Parasitology **89:** 553–564.

INGHAM, R. E., P. B. HAMM, M. BAUNE, N. L. DAVID, AND N. M. WADE. 2007. Control of *Meloidogyne chitwoodi* in potato with shank-injected metam sodium and other nematocides. Journal of Nematology **39:** 161–168.

JENSEN, J. B., P. A. NYBERG, S. D. BURTON, AND W. R. JOLLEY. 1976. The effects of selected gases on excystation of coccidian oocysts. Journal of Parasitology **62:** 195–198.

JOHNSON, J. K., AND W. M. REID. 1970. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chicken. Experimental Parasitology **28:** 30–36.

JOHNSTON, W. T., M. W. SHIRLEY, A. L. SMITH, AND M. B. GRAVENOR. 2001. Modeling host cell availability and the crowding effect in *Eimeria* infections. International Journal for Parasitology **10:** 170–1081.

KIELY, T., D. DONALDSON, AND A. GRUBE. 2004. Pesticides industry sales and usage: 2000 and 2001 market estimates. *In* 2000–2001 pesticide market estimates biological and economic analysis. Division Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC., p. 1–48.

KING, B. J., AND P. T. MONIS. 2007. Critical processes affecting *Cryptosporidium* oocysts survival in the environment. Parasitology **134:** 309–323.

MATHIESSEN, J. N., AND M. A. SHACKLETON. 2005. Biofumigation: Environmental impacts on the biological activity of diverse pure and plant derived isothiocyanates. Pesticide Management Science **61:** 1043–1051.

SHIRLEY, M. W., A. SMITH, AND D. P. BLAKE. 2007. Challenges in the successful control of the avian coccidia. Vaccine **25:** 540–547.

TILTON, F., AND R. L. TANGUAY. 2008. Exposure to sodium metam during zebra fish somitogenesis results in early transcriptional indicators of the ensuing neuronal and muscular dysfunction. Toxicology Science **106:** 121–127.

WAINWRIGHT, K. E., M. LAQUNAS-SOLAR, M. A. MILLER, B. C. BARR, I. A. GARDNER, C. PINA, A. C. MELLI, A. E. PACKHAM, N. ZENG, T. TRUONG ET AL. 2007. Physical inactivation of *Toxoplasma gondii* oocysts in water. Applied Environmental Microbiology **73:** 5663–5666.